



Two new species of *Graphis* (Ascomycota: Ostropales: Graphidaceae), from the Indo-Burma biodiversity hotspot

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Abstract

Graphis manipurensis and *Graphis sirohiensis* are described as new lichenized fungi from Manipur in the Indo-Burma biodiversity hotspot. *Graphis manipurensis* is characterized by its very short and unbranched lirellae (*cleistomma*-morph), entire labia, laterally carbonized excipulum, heavily inspersed hymenium, 1-spored asci with muriform ascospores and presence of norstictic acid. *Graphis sirohiensis* is characterized by its prominent lirellae (*marginata*-morph), entire labia, completely carbonized excipulum, heavily inspersed hymenium, 8-spored asci with submuriform to muriform ascospores and presence of norstictic acid.

Key words – Graphidaceae, Manipur, new species, lichens, taxonomy

Introduction

The state of Manipur in the north east India and part of the Indo-Burma biodiversity hotspot (Myers *et al.* 2000) is an interesting area for lichen research. Müller (1892) was the first worker who described 29 new taxa out of 101 taxa now reported from the state. Subsequently, some more publications were made by Singh (1980a, b, c, 1981a, b, 1982, 1983, 1984, 1986) from the same area. While studying Graphidaceae collections from Manipur (Fig. 1), two new species of *Graphis* were discovered. The genus as circumscribed by Staiger (2002), Lücking (2009) and Lücking *et al.* (2009) is characterized by its crustose thallus with trentepohlioid photobiont; simple to branched, rounded to lirellate or rarely pseudostromatic ascomata with apically, laterally or completely carbonized exciple; clear or inspersed hymenium, I– with simple paraphyses; nonamyloid, unitunicate 1–8 spored asci with apically thickened wall; and hyaline, transversely septate to muriform and amyloid ascospores with lens shaped lumina. It is widely distributed in tropical and subtropical regions, with at least one species in temperate regions, and represented by 125 species (Singh & Sinha 2010, Chitale *et al.* 2011, Singh & Swanlatha 2011 a, b) in India out of *c.* 368 species known from the world (Lücking *et al.* 2009; Bárcenas-Peña *et al.* 2014). Two further new species *Graphis manipurensis* and *Graphis sirohiensis* -are described below.

Materials & Methods

Specimens collected from Manipur and deposited in CAL herbarium were investigated. Morphological observations were made using a stereomicroscope (Nikon SMZ 1500). Thin hand-

cut sections of thalli and ascomata were mounted in water, lactophenol cotton blue (LPCB), 10% KOH and Lugol's iodine solution. All anatomical measurements were taken in water mounts and examined under a compound microscope (Nikon Eclipse 50i). Lichen substances were identified by thin layer chromatography (TLC) in solvent A (180 Toluene: 60 dioxane: 8 acetic acid) following White & James (1985).

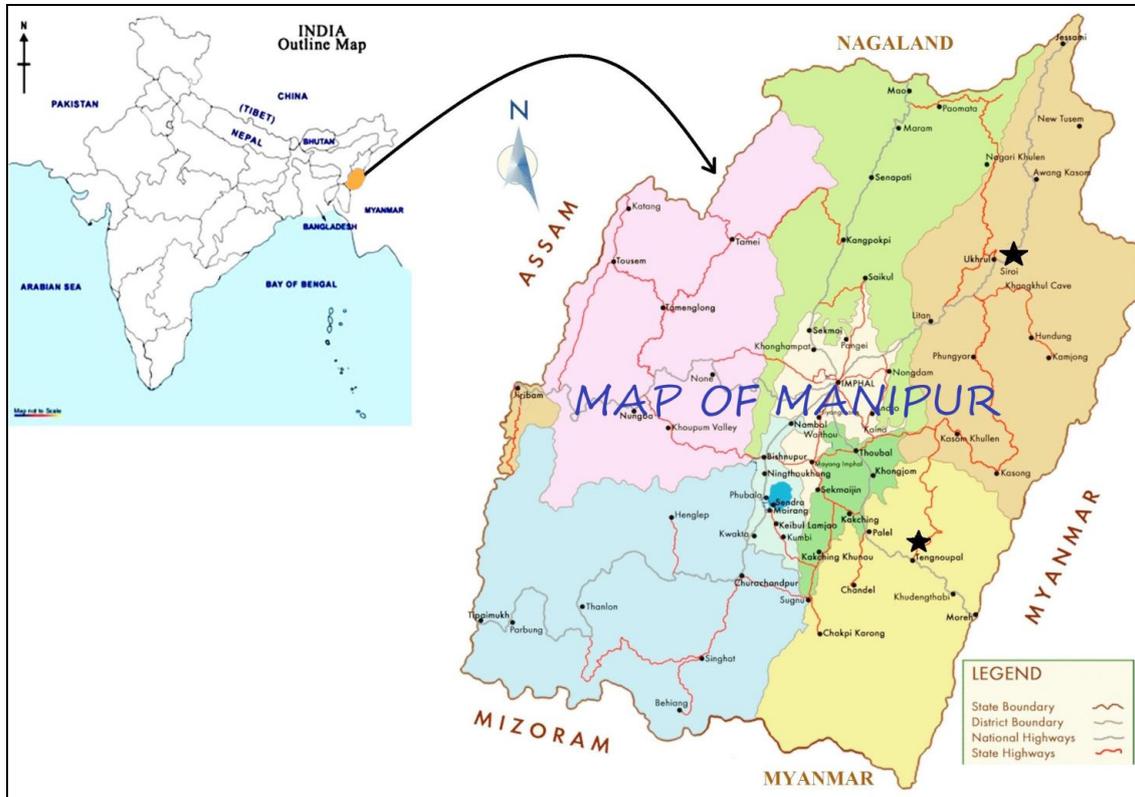


Fig 1. Study area, Manipur state, India

1. *Graphis manipurensis* Pushpi Singh & Kr. P. Singh, sp. nov.

Fig. 2 A–E

MycoBank: MB 809433

Etymology – The species epithet refers to the state from where the new species is being described.

Holotype: India, Manipur, Chandel district, Tengnoupal, 24° 38' 2'' N; 94° 15' 2'' E, on the bark of unidentified phorophyte, alt. c. 1550 m, K.P. Singh 550923 (Holotype–CAL).

Differing from Graphis nadurina in the larger ascospores, from G. cleistomma and G. oryzaearpa in the norstictic acid chemistry, and from G. insulana in the lirellae morphology.

Thallus corticolous, crustose, epiphloeodal, greyish brown to reddish brown, continuous, ± rugulose, 3–6 cm across, 100–175 µm thick; cortex indistinct; algal cells compactly arranged forming a layer like zone, 40–60 µm thick, below the algal layer patches of large hyaline crystals present; photobiont *Trentepohlia*, algal cells 8–10 µm across; prothallus indistinct.

Apothecia lirelliform, very short and unbranched (*cleistomma*-morph), straight, erumpent to prominent, acute to obtuse at ends, apically covered by thick complete thalline margin, 0.8–1.4 mm long, 0.55–0.8 mm wide, 0.3–0.42 mm high; disc concealed; thalline margin concolourous with the thallus, encrusted with large clusters of calcium oxalate crystals; labia entire, convergent; exciple

open to slightly closed at base, laterally carbonized, 80–110 μm thick, together with thalline margin 140–180 μm thick; epihymenium pale brown, 10–15 μm high; hymenium hyaline, heavily inspersed, 170–210 μm high, I–; paraphyses simple, dense, 1–1.5 μm thick; asci 1-spored, cylindrical, 130–180 \times 24–30(–35) μm , I–; ascospores colourless, ellipsoid, densely muriform, transversely (12–) 20–24 septate, longitudinally (3–)10–12 septate, (98–)100–135 \times (20–)25–45 μm , I+ violet blue.

Chemistry – Thallus K+ yellow forming red crystal, C–, P–; UV–. TLC: Norstictic acid (major) present.

Remarks – The species is characterized by its greyish brown to reddish brown thallus, very short and unbranched lirellae (*cleistomma*-morph), completely covered by thick thalline margin, laterally carbonized excipulum with entire labia, strongly inspersed hymenium, 1-spored asci with large muriform ascospores and presence of norstictic acid. Morphologically, it closely resembles *Graphis nadurina* Aptroot, *G. cleistomma* Nyl. and *G. oryzaecarpa* Lücking, which all have clear hymenia. Further, *G. nadurina* differs in having smaller ascospores (40–50 \times 14–16 μm , vide Lücking *et al.* 2009) whereas *G. cleistomma* and *G. oryzaecarpa* have a completely carbonized excipulum and lack lichen substances. Anatomically, it closely resembles *Graphis insulana* (Müll. Arg.) Lücking but differs in the lirellae morphology. The new species is known only from the type locality, where it grows on the bark of tree in open places.

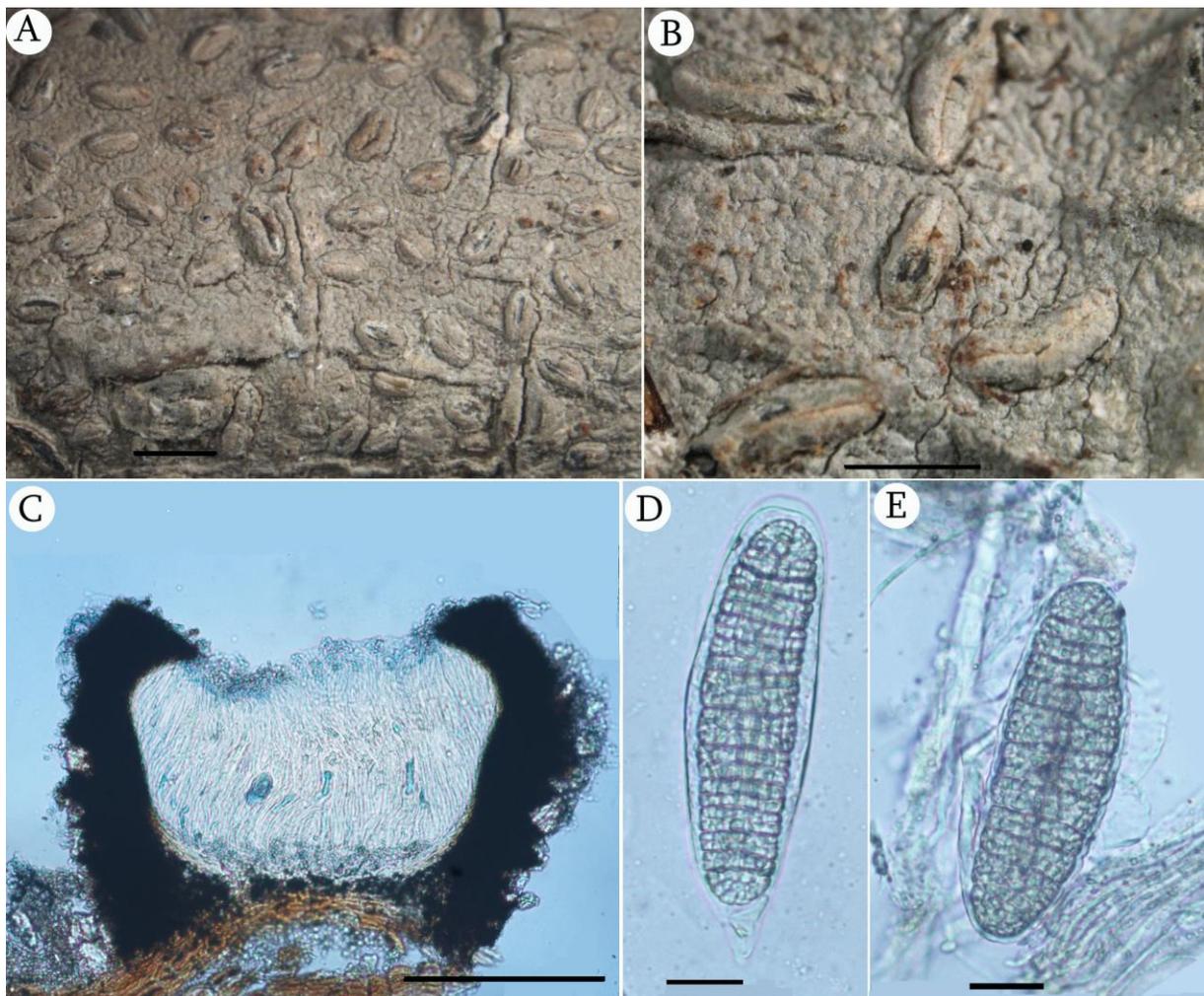


Fig. 2 A–E – *Graphis manipurens*. A–B, habit; C, cross section of apothecium (in LPCB); D, ascus; E, mature ascospores. – Bars = A & B=1 mm; C=200 μm ; D & E=25 μm .

2. *Graphis sirohiensis* Pushpi Singh & Kr. P. Singh, **sp. nov.**

Fig. 3 A–E

MycoBank: MB 809435

Etymology – The species epithet refers to the name of the type locality from where the new species is collected.

Holotype: India, Manipur, Ukhrul district, 25° 6' 21.58'' N; 90° 27' 25.89'' E, on way to Sirohi hill, on the bark of tree, alt. c. 1650 m, K.P. Singh 54810 (holotype – CAL).

Differing from Graphis saxicola in the corticolous habit and has prominent lirellae with thin thalline margin.

Thallus corticolous, crustose, epiphloeodal, pale brown to yellowish brown, continuous, smooth to ± rugulose, 4–6 cm across, 40–60 µm thick; cortex indistinct; photobiont layer 30–45 µm thick; photobiont *Trentepohlia*, algal cells 6–8 µm across; µm thick; prothallus indistinct.

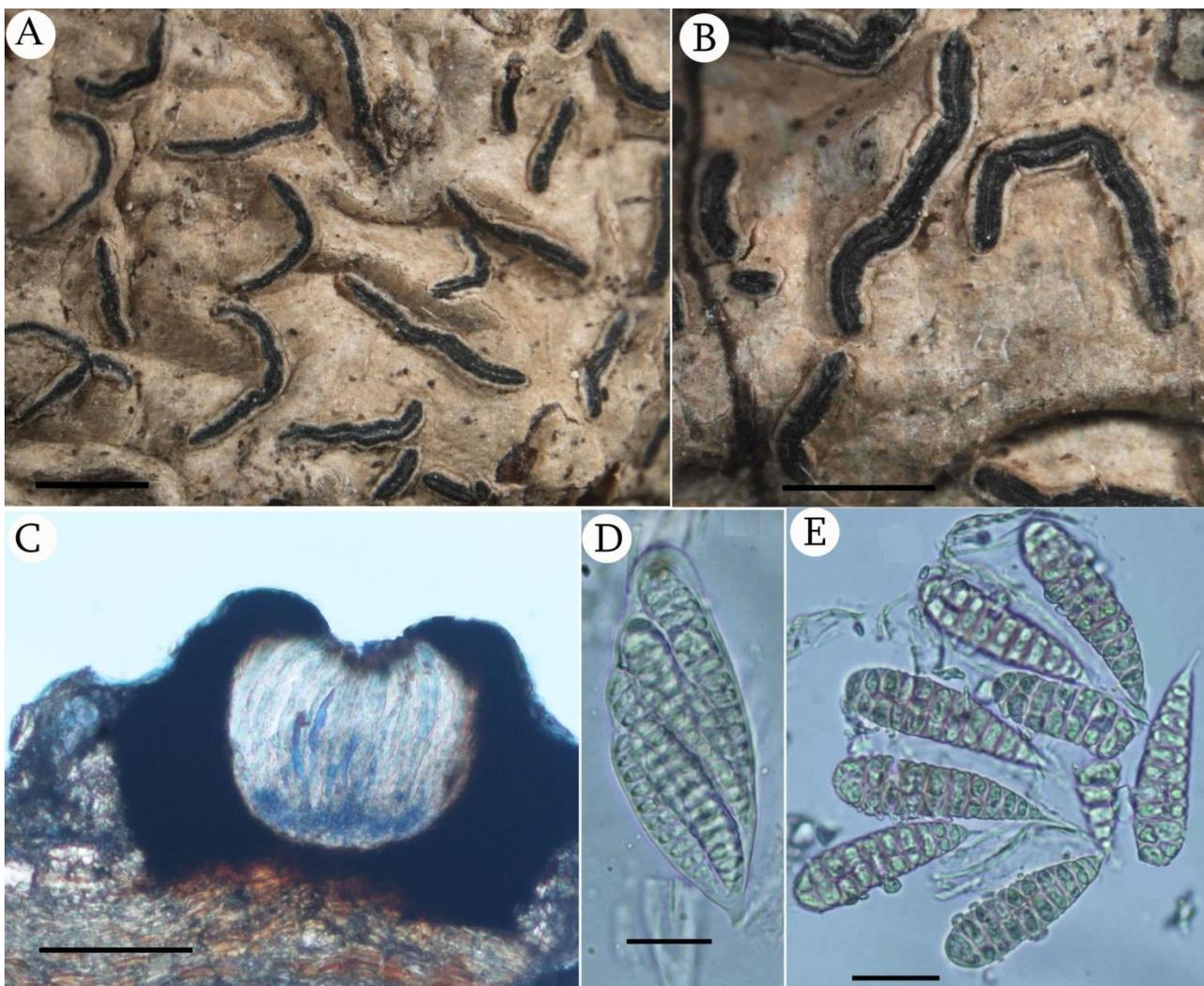


Fig. 3 A–E – *Graphis sirohiensis*. A–B, habit. C, cross section of apothecium (in LPCB). D, ascus. E, mature ascospores. – Bars = A & B=1 mm; C=200 µm; D & E=20 µm.

Apothecia lirelliform, black, unbranched to rarely triradiate, straight to ± flexuose, prominent (*marginata*-morph); acute to obtuse at ends, laterally covered by thin thalline margin, 1.2–3 mm long, 0.35–0.56 mm wide, 0.25–0.42 mm high; disc concealed to partly exposed, blackish, epruinose; thalline margin concolourous with the thallus, encrusted with large clusters of

calcium oxalate crystals; labia entire, convergent; exciple basally slightly open to closed, completely carbonized, 50–90 µm thick, together with thalline margin 95–160 µm thick; epihymenium pale brown, 8–15 µm thick; hymenium hyaline, heavily inspersed, 150–200 µm high, I–; paraphyses simple, dense, slightly clavate, pale brown at apices, 1–1.5 µm thick; asci 8-spored, clavate to cylindrical with biserially arranged ascospores, 98–120(–140) × 24–30(–35) µm, I–; ascospores colourless, clavate, one end rounded and other end becoming narrower, submuriform to muriform, transversely (7–)10–11 septate, longitudinally 0–2 septate, (35–)40–51 × 10–13 µm, I+ violet blue; young ascospores with thick episore.

Chemistry: Thallus K–, C–, P–; UV–. TLC: Norstictic acid (minor) present.

Remarks: *Graphis sirohiensis* is well characterized by its smooth, yellowish brown to pale brown thallus, prominent lirellae (*marginata*-morph) with laterally thick thalline margin, partly exposed disc, completely carbonized excipulum with entire labia, heavily inspersed hymenium, small, clavate, submuriform to muriform ascospores and presence of norstictic acid. Morphologically, anatomically and in chemistry, new species closely resembles *Graphis handelii* Zahlbr. and *G. crebra* Vain., but both later species differ in having transversely septate ascospores. In addition, *G. crebra* differs in having white pruinose disc. In general appearance, it also somewhat resembles *G. ficicola* Vain. and *G. palmicola* Makhija & Adaw., which have clear hymenia, transversely septate ascospores, and different chemistry. It also resembles *G. saxicola*, in anatomy and chemistry and spore size, but differs in saxicolous habit and has erumpent lirellae with thick lateral thalline margin. The new species is known so far only from the type locality where it grows at an altitude of c 1650 m on the bark of tree in open shady places.

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